Study of *EZH2* Gene Expression in Pediatric Myelodysplastic Syndrome

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INTRODUCTION

Myelodysplastic Syndrome (MDS) is a heterogeneous group of clonal diseases of hematopoietic stem cells, characterized by the presence of dysplasias and cytopenias in the peripheral blood. Recent discoveries at the molecular level have provided new insights related to the development of MDS and its evolution to acute myeloid leukemia (AML). Studies have shown that the gene of the Polycomb family, *Enhancer of Zeste Homolog 2 (EZH2)*, may be a strong candidate involved in the pathogenesis of MDS. This gene plays a critical role in the normal development of the hematopoietic system and it is involved in the maintenance of the hematopoietic stem cell population. The *EZh2* is involved in various mechanisms associated with cancer (Figure 1).

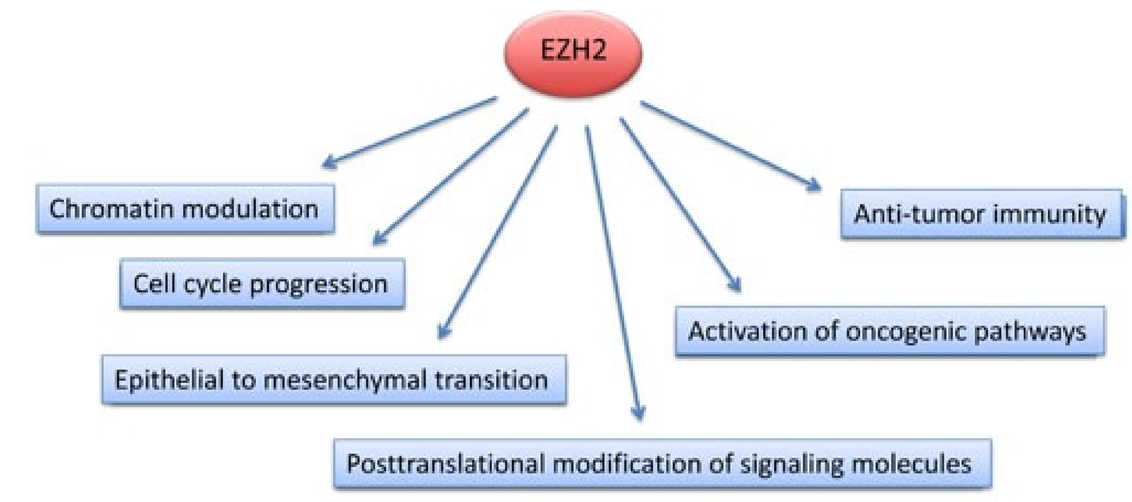

Figure 1: Mechanisms of *EZH2*-mediated implications in cancer (Chistofides et al, Oncotarget, 2016).

Polycomb Group (PcG) proteins have been established as classical players of epigenetic regulation. PcG proteins contain two core complexes: the maintenance polycomb repressive complex 1 (PCR1) and the initiation polycomb repressive complex 2 (PCR2) (Figure 2). The *Enhancer of Zeste Homolog 2* (*EZH2*) is a histone methyltransferase. It is the catalytic subunit of PCR2 for tri-methylation of histone 3 at lysine 27 (H3K27me) by SET domain in its C-terminus, which silences target genes involved in various biological functions as cell cycle, cell proliferation and differentiation. To date, few studies have evaluated the expression of *EZH2* in adult patients with MDS. However, until now there are no studies showing the expression of *EZH2* in pediatric patients with MDS. So, the aim of this study was to analyze the expression pattern of the *EZH2* gene in pediatric patients with MDS and its association with the evolution from MDS to AML.

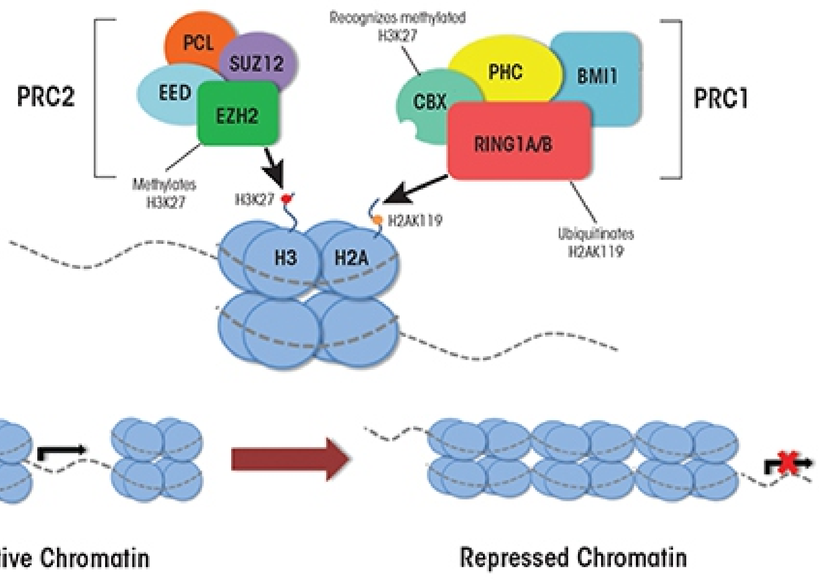

Figure 2: Polycomb repressive complex 1 (PRC1) consists of four core proteins including: polyhomeotic homolog (PHC), ring finger protein 1A or 1B (RING1A or RING1B), B-lymphoma Mo-MLV insertion region 1 homolog (BMI1), and chromobox homolog (CBX). The RING1A/RING1B subunits are the catalytic engine of the PRC1 complex and carry out ubiquitination of histone 2A at lysine 119 (H2AK119ub). PRC2 consists of four core proteins including: embryonic ectoderm development (EED), enhancer of zeste 2 (EZH2), suppressor of zeste 12 (SUZ12), and polycomb like (PCL). EZH2 serves as the catalytic subunit of PRC2 and trimethylates lysine 27 on histone 3 (H3K27me3). Current models suggest that H3K27me3 generated by PRC2 facilitates compaction of chromatin leading to the repression of gene expression (Veazey *et al*, *Alcohol Research: Current Reviews*, 2010).

PATIENTS AND METHODS

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Pacientes n=42, 27 boys and 15 girls, mean of age: 8 years
17 healthy pediatric controls, bone marrow donors, mean of age:12 years

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RNA extraction - bone marrow cells (Trizol)/DNAse /cDNA

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Real-time quantitative polymerase chain reaction (qPCR),
Rotor Gene Q thermocycler (Qiagen)

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45 cycles: 20 seconds at 95°C, 30 seconds at 60°C and 30 seconds at 72°C
***B-actin* mRNA levels were used as a reference for normalization**

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Statistical analysis: association with *EZH2* expression and evolution from MDS to AML

RESULTS

We observed that the relative expression levels of the *EZH2* gene in 42 pediatric patients were higher than the pediatric healthy donors ($p<0.04$) (Mann-Whitney test) (Figure 3). The evolution from MDS to AML was observed in 38% of patients (16/42). We observed a statistical significance between the *EZH2* relative level of expression and the evolution of the disease ($p<0.001$).

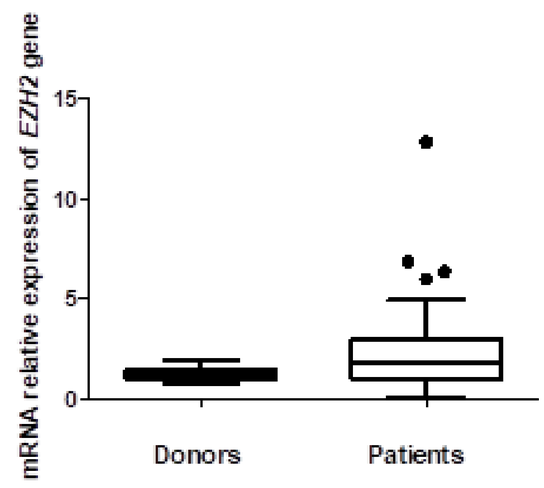

Figure 3: Analysis of mRNA relative expression of *EZH2* gene: healthy donors and pediatric MDS patients.

CONCLUSION

To our knowledge, this is the first study showing the expression of *EZH2* in pediatric patients with MDS and its association with disease evolution. Aberrant expression of *EZH2* gene in MDS is a possible biomarker of disease evolution and it is associated with poor prognosis.

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